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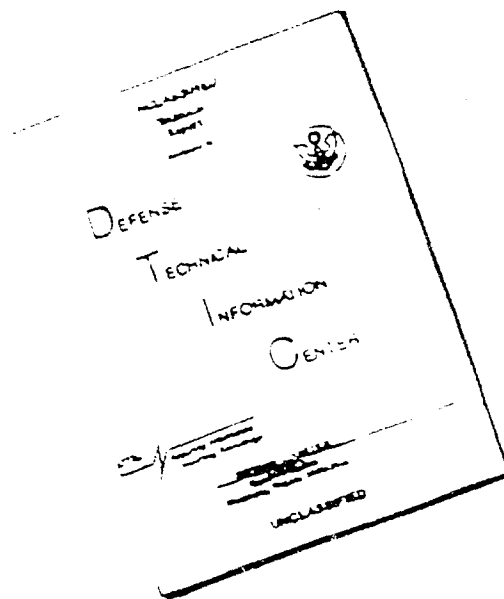
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INFECTIOUS PROCESS IN
WHITE MICE INFECTED WITH
PATHOGENIC MICROORGANISMS
plus SUMMARY**

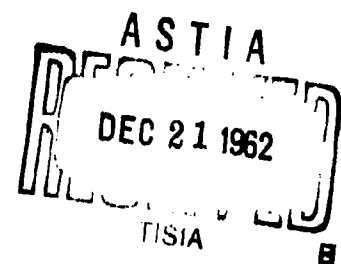
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**Effect of Cortisone on the Course of the Infectious Process in
White Mice Infected with Pathogenic Microorganisms¹⁾**

By G. V. Yushchenko, M. P. Tereshchenko, R. V. Kovaleva,
T. N. Ponomareva, N. S. Ogneva, and L. V. Rodkevich

Central Antiplague Observation Station of the
Ministry of Health USSR

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pages 134-138

It is considered as an established fact that cortisone reduces the natural resistance of the organism of infections by inhibiting its protective mechanisms. In animals which had received cortisone the course of infection is characterized by a greater dissemination of microorganisms within the organism, reduction of the inflammatory reaction, and a higher lethality rate (for summary of the data see Zil'ber, 1958). An opinion has been advanced that white mice treated with cortisone can be employed for biotests in examinations for the presence of the plague infection, especially in cases where the causative agent is contained in the material in small quantities or possesses low virulence (Blyakher, 1958; Ter-Vartanov and coauthors, 1959).

Shtel'man (1960) employed cortisone-treated white mice for the elicitation of experimental plague infection in the organism of meridional gerbils. Upon examination of gerbils infected with a highly virulent strain of *Past. pestis* (Dolm for guinea pigs comprised 100 microbial cells), only a slight shortening of death periods was observed in the experimental animals, as compared with the death of control mice which had not been treated with cortisone. Upon infection of gerbils with a less virulent strain (Dolm for guinea pigs equals 500 microbial cells), the number of positive findings in the experimental animals was somewhat higher than in controls which had not been treated with cortisone. However, one case was observed where the culture could be isolated only from a white mouse which had not received cortisone. The author points out that seedings on the nutritive media of the organs of perished and killed cortisone-treated mice grew an alien microflora more often than in the case of control animals.

Kozakevich and coauthors (1960) noted an increased sensitivity to plague infection in susliks treated with cortisone. Braude reported an

1) The work was carried out under the direction of N. G. Olsuf'yev and reported on 9 April 1960 at the Scientific Conference of the Central Antiplague Station.

increase of sensitivity to brucellae of cortisone-treated white mice (1960).

We set ourselves the task of corroborating experimentally on white mice the cortisone effect in a number of bacterial infections, in order to ascertain in what cases its administration to animals for a biological test may improve or accelerate the laboratory diagnosis of these infections.

The strains of causative agents of plague, tularemia, pseudotuberculosis, pastereiosis, listerellosis, erysipeloid, and mouse paratyphoid were used in this investigation. We employed not only recently-isolated fully virulent strains, but also strains with reduced virulence as well as artificially attenuated cultures.

Tests were carried out on white mice weighing 16 to 18 grams. In the processing of mice we used a French preparation of cortisone acetate of the Roussel firm. According to the plan suggested by Blyakher, we determined the optimal cortisone dose of five mg which corresponded to 0.2 ml of the preparation. Cortisone was administered to the animals intramuscularly four hours prior to the experiment. At the end of this period the animals received subcutaneously a suspension of a 48-hour culture of tularemic or plague microorganisms, or a diurnal (18 to 24 hours) culture of other causative agents in various doses, depending on the virulence of the strain. Each dose was usually given to a group of five animals. Similar doses were injected simultaneously to control animals (i.e., not treated with cortisone).

The density of the initial suspension was determined according to the optic standard of the State Control Institute, and were subsequently diluted 10, 100, etc., times. As control of the correct dilution of the cultures, served seedings of low dilutions (1, 10, and 100 microbial cells) on artificial nutritive media, with the count of grown colonies. The tularemic cultures were excepted, since for the purpose of control they had not been seeded.

The diagnosis on perished animals was made on the basis of patho-anatomical changes in the internal organs, results of bacterioscopic examination, and isolation of the initial culture by means of seeding. Observation of experimental animals was carried out for three to four weeks, after which the survived animals were killed, dissected, and their internal organs examined.

A total of 20 strains of various bacterial species was investigated; 1163 white mice were used in the experiments.

Upon testing of four strains of *Past. pestis* recently isolated from rodents at the infection nidus, only slight variations were noted in the number of plague-killed white mice which had been treated with cortisone as compared to the untreated ones. Of 40 animals of the first group,

infected with doses of one and ten microbial cells of the above-mentioned strains, 19 died of plague, and from the same number of animals of the second group -- 14 died (Table 1). This difference cannot, however, be considered reliable since, upon testing with No 2212 and No 321 strains, less experimental mice perished than controls. We did not use the dose of 100 microbial cells in these experiments (with the exception of strain No 926), because it was found that it was absolutely lethal to white mice even without cortisone.

Upon seeding of dishes of nutritive media with a suspension containing (as per calculation) ten microbial cells, two to six colonies grew, and upon seeding with a suspension of 100 microbial cells -- 26 to 41 colonies of the plague bacillus were counted.

Table 1

Effect of cortisone upon infection of white mice with virulent or vaccine strains of *Past. pestis*

Number of microbial cells in the dose, upon infection	Virulent strains								EV vaccine strains	
	No 926		No 2212		No 321		No 2056		without corti- sone	with corti- sone
	without corti- sone	with corti- sone	without corti- sone	with corti- sone	without corti- sone	with corti- sone	without corti- sone	with corti- sone		
1	5/2	5/3	5/1	5/1	5/1	5/0	5/0	5/2	—	—
10	5/2	5/5	5/3	5/2	5/5	5/4	5/0	5/2	—	—
100	5/5	5/5	—	—	—	—	—	—	5/0	2/0
1 000	—	—	—	—	—	—	—	—	5/0	5/2
10 000	—	—	—	—	—	—	—	—	5/1	4/2
100 000	—	—	—	—	—	—	—	—	5/0	4/4
1 million	—	—	—	—	—	—	—	—	5/1	5/5
10 "	—	—	—	—	—	—	—	—	4/2	5/5
100 "	—	—	—	—	—	—	—	—	5/5	5/5
Total	15/9	15/13	10/4	10/3	10/6	10/4	10/0	10/4	34/9	30/23

Designations: numerator -- number of experimental animals; denominator -- number of perished animals (the animals which had died of other causes are excepted).

As regards the time of death of the mice, upon inoculation of one and ten cells, no shortening of its period was observed in cortisone-treated animals; after inoculation with one microbial cell the experimental mice died within four to nine days, controls -- within three to six; after a dose of ten microbial cells they died, respectively, within four to ten and four to seven days. But after a dose of 100 microbial cells the experimental animals died a day sooner than controls.

These data indicate that white mice were highly sensitive to the virulent strains of *Past. pestis* recently isolated from the infection nidus, and that cortisone had no substantial effect on the results.

Table 2

Effect of cortisone, upon infection of white mice with virulent or vaccine strains of *Pasteurella tularensis*

Number of microbial cells in the dose, upon infection	Virulent strain No 9		15-reduced vaccine strain	
	without cortisone	with cortisone	without cortisone	with cortisone
0.1	15/11	13/10	—	—
1	10/10	10/10	5/0	5/1
10	—	—	5/1	5/5
100	—	—	5/1	5/4
1 000	—	—	5/3	5/5
10 000	7	—	5/2	5/5
100 000	—	—	5/4	5/5
1 million	—	—	5/2	5/5
Total . . .	25/21	23/20	35/13	35/30

An appreciable increase of sensitivity under the effect of cortisone was observed upon testing of the plague vaccine EV strain, which coincides with the data of Payne and Larson (1955), Girard (1957), Blyakher (1958), et al. In our tests, upon infection of white mice with doses from 100 to 100 million microbial cells, of 30 experimental mice 23 died, whereas of 34 controls -- only nine. The experimental mice died, on the average, two days sooner than controls.

Upon testing of a fully virulent strain of *Past. tularensis* (No 9), no increased sensitivity of white mice was noted following administration of cortisone. The number of tularemia-killed animals, upon their infection with 0.1 and one microbial cell, proved to be identical in the experimental and control groups (Table 2).

It should only be noted that cortisone-treated animals died, on the average, a day sooner than controls. Upon inoculation with the 15-reduced Gaytskiy vaccine strain, a marked increase of sensitivity was elicited in cortisone-treated mice, as compared with the untreated ones;

death of the animals from doses of one to one million microbial cells was summarily twice as high in the first group than in the second one, and the experimental animals died a few days earlier than controls. Four pseudotuberculous strains were then tested, including two virulent (recently-isolated from wild rodents), one -- with reduced virulence, and one -- an avirulent strain. In all cases a noticeable increase of sensitivity of white mice was observed under the effect of cortisone (Table 3). Upon testing of a fully virulent strain No 1144, all experimental animals perished even from a dose of ten microbial cells, whereas in the control group the smallest absolutely lethal (D₅₀) comprised 100,000 microbial cells. Strain No 2218 with reduced virulence caused in corresponding doses (from one to 100 million microbial cells) the death of 80% of experimental animals as against 12% in the control group. Cortisone-treated mice died of pseudotuberculosis faster than controls. In particular, upon administration of such doses as 10,000 or 100,000 microbial cells, the difference in the death periods reached three to seven days. From the avirulent strain No 2413 in the control group all mice survived, including those inoculated with a dose of one billion microbial cells, whereas of 45 experimental mice 11 died of pseudotuberculosis.

Table 3

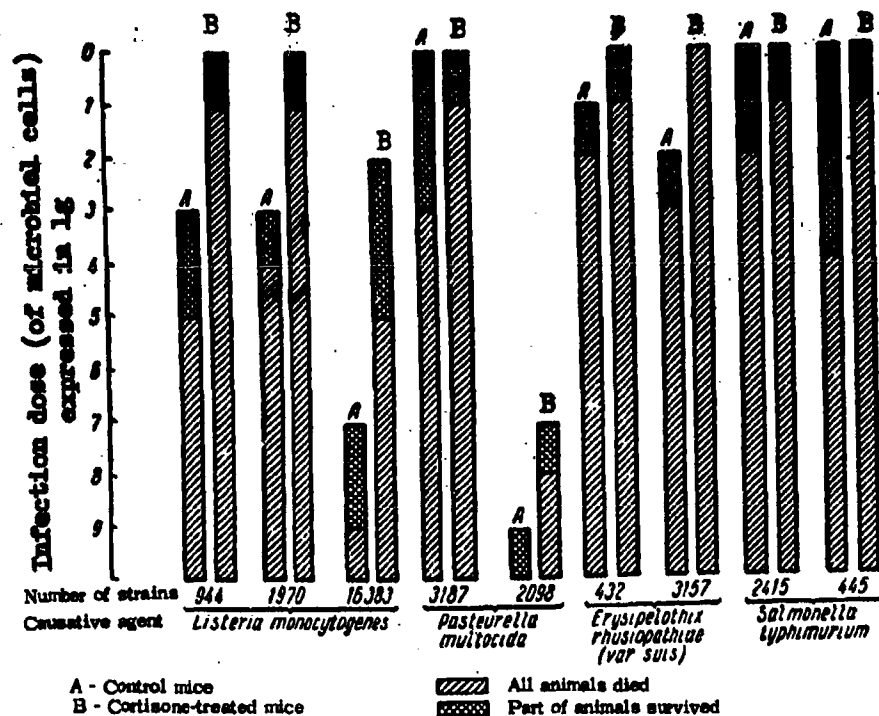
Effect of cortisone upon infection of white mice
with strains of the pseudotuberculous bacillus
of various degrees of virulence

Number of microbial cells in the dose, upon infection	Virulent strains				Strain with reduced virulence No 2218		Avirulent strain No 2413	
	No 1144		No 423		without corti- sone	with corti- sone	without corti- sone	with corti- sone
	without corti- sone	with corti- sone	without corti- sone	with corti- sone				
1	5/0	5/2	3/1	5/1	5/0	5/0	5/0	5/0
10	5/3	5/5	3/0	5/3	5/0	1/0	5/0	5/0
100	5/3	5/5	3/1	5/5	4/0	3/3	5/0	5/0
1000	4/3	5/5	3/1	5/4	5/0	5/4	5/0	5/1
10000	5/3	5/5	3/3	5/5	5/1	5/4	5/0	5/0
100000	5/5	5/5	3/2	5/5	5/1	4/4	5/0	5/4
1 million	5/5	5/5	3/2	5/5	4/1	5/5	5/0	5/1
10 "	5/5	5/5	3/3	5/5	4/0	5/5	5/0	5/3
100 "	5/5	5/5	3/3	5/5	5/2	5/5	5/0	5/2
Total . . .	44/32	45/42	27/16	45/38	42/5	38/30	45/0	45/11

Designations: the same as in Table 1.

The authors also tested three *Listeria* strains, two *Past. multocida* strains, two strains of *erysipelotrix* (variant suis), and two *salmonella* strains. The majority of the strains were fully virulent, and were used shortly after their isolation from rodents caught in the city. But one *Listeria* strain (No 16383) was of reduced virulence to white mice, and one *Past. multocida* strain (No 2098) was almost avirulent. The results of tests with these causative agents were identical with the results obtained with the pseudotuberculosis micro-organism. In all cases, an increased sensitivity of mice to infection was observed following administration of cortisone, which could be judged by the change in the minimal lethal dose (Dlm) and minimal absolutely lethal dose (Dolm) of a given causative agent injected to the animals. Thus, for instance, upon testing on cortisone-treated mice of the fully virulent strains of any employed agents, the Dlm comprised only one microbial cell, and Dolm -- ten microbial cells (upon testing of No 3157 strain of *erysipelotrix*, the Dolm proved to be equal to one microbial cell). In comparison with the test data on control animals, it represented a reduction of corresponding lethal doses equalling ten or 100 fold, and in some cases -- 1000 and 10,000 fold (*Listeria* strains No 944 and 1970). Upon testing of a *Listeria* strain with reduced virulence, its Dlm under cortisone processing decreased from ten million to 100 microbial cells, and Dolm -- from one billion to 100,000 microbial cells. Hence, cortisone-treated mice became as sensitive to this strain, as control mice to the fully virulent *Listeria* strains. The nearly avirulent *Past. multocida* strain No 2098 caused the death of cortisone-treated mice following injection of one billion and 100 million microbial cells, whereas part of the animals perished from ten million microbial cells; in the control group only two mice out of five died after a dose of one billion, and all animals which had received smaller doses survived. The death periods of mice from listerellosis, erysipeloid, and other infections were shorter under the effect of cortisone; this was particularly noticeable when moderate and small doses (1000 to 100,000 microbial cells) of listerella and salmonella cultures had been administered.

Thus, cortisone considerably increased the sensitivity of mice to infections to which these animals, when not treated with cortisone, had manifested a moderate or reduced sensitivity. Their increased sensitivity to these infections was expressed not only in the reduction of the Dolm, but also in the shortening of the period of dissemination of their organs and tissues with the corresponding causative agents -- a fact which facilitated and accelerated the laboratory diagnosis. All this indicates the expediency of the use of cortisone for the processing of mice employed in biological tests during examinations for listerellosis, pseudotuberculosis, and other infections to which white mice do not manifest an absolute sensitiv-



In analogous manner cortisone markedly increased the sensitivity of white mice to the vaccine strains of the plague and tularemia microorganisms and, hence, in a number of cases this preparation can be used in experimental work.

As regards the fully virulent cultures of plague and tularemia agents to which white mice manifest very high sensitivity even without cortisone, the administration of this preparation in our experiments either caused no rise in the lethality rate (tularemia), or slightly modified it (plague).

In order to ascertain the expediency of using cortisone-treated white mice in biological tests for plague of rodents under natural conditions, we carried out corresponding control tests in 1958 in Guryevskaya Oblast (Kovaleva and Ponomareva). The work was carried out in the plague nidus during an epizooty among rodents. Animals, caught in traps or snared, were examined. In the majority of cases they were gerbils (*Rhombomys opimus*), and less frequently -- house mice (*Mus musculus*), in a few cases -- small susliks (*Citellus pygmaeus*). The material (pieces of spleen and liver) from the rodents was triturated, weighed and then injected in a physiological solution

subcutaneously into a white mouse which had been treated with cortisone (according to the same method which has been used in the above-described experiments), and simultaneously into another mouse which had not been given cortisone. We were unable to follow the suggestion of various authors (Blyakher, 1958; Ter-Vartanov and coauthors, 1959) of using for each analysis five to ten cortisone-treated mice, since it was impossible to carry it out technically under conditions of practical work in the epidemic focus. Neither did we use the intravenous method of the injection of the material, as suggested by the same authors, because this would have considerably increased the death rate of the animals from an alien flora, since the material for examination was not always supplied in a fresh state.

A total of 151 double biotests were carried out during the period from April to July. Six mice untreated and five treated with cortisone died of plague. Cultures of the causative agent were isolated from all animals which had died from plague. It should be pointed out that the seedings from dead cortisone-treated mice were often contaminated with alien microorganisms -- a fact which somewhat hampered the biotests. These data coincide with Shtel'man's observations (1960). In all our analyses the death from plague of cortisone-treated mice coincided with the death of the untreated ones, with the exception of one case when a cortisone-treated mouse survived, and an untreated one died of plague on the 7th day. The death periods of the cortisone-treated mice were: in one case identical to the death period of the control animal; in three cases they were ahead by one to two days, and in one it was retarded by 24 hours. Positive results were obtained in three cases from large gerbils and in three -- from house mice.

As a result of these examinations, no particular advantages were elicited of the laboratory diagnosis of plague in wild rodents upon the use of cortisone-treated white mice for biological tests.

Conclusions

1. Under experimental conditions cortisone considerably increased the sensitivity of white mice to pseudotuberculosis, listerellosis, pastorellosis, erysipeloid, and salmonellosis; therefore, cortisone-treated white mice can be correspondingly employed as a very sensitive biotest in carrying out corresponding analyses.
2. Cortisone markedly increased the sensitivity of white mice to the vaccine culture of plague and tularemia microorganisms and thus can be utilized in individual experimental investigations.
3. In experiments with highly virulent cultures of tularemia and plague microorganisms, to which white mice are highly sensitive even without cortisone, no further increase has been observed in the sensitivity of animals to infection (tularemia), or their sensitivity in-

creased only slightly (plague).

4. Upon examination of wild rodents in their natural plague nidi, no advantages could be elicited from the use of cortisone-treated mice for biological tests.

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E N D

SUMMARY

Effect of Cortisone on the Course of the Infectious Process in White Mice Infected with Pathogenic Microorganism¹⁾

By G. V. Yushchenko, M. P. Tereshchenko, R. V. Kovaleva,
T. N. Ponomareva, N. S. Ogneva, and L. V. Rodkevich

Central Antiplague Observation Station of the
Ministry of Health USSR

(Received by editors 19 April 1960)

It is considered as an established fact that, in inhibiting the protective mechanisms, cortisone reduces the natural resistance of the organism to infections. In animals which had received cortisone the course of the infection is characterized by greater dissemination of the microorganisms within the organism, reduction of the inflammation reaction, and a higher lethality. An opinion has been advanced that white mice treated with cortisone can be used for biotests in examinations for the presence of plague infection, especially in cases where the causative agent is found in small quantities in the tested material or possesses low virulence.

The authors set themselves the task of verifying experimentally on white mice the effect of cortisone in a number of bacterial infections, in order to clarify the question as to the cases where its administration to biologically tested animals may improve or accelerate the laboratory diagnosis of these infections.

The strains of causative agents of plague, tularemia, pseudotuberculosis, pasteurellosis, listerellosis, erysipeloid, and mouse paratyphoid were employed in this investigation. Recently-isolated, fully virulent strains were used, as well as strains with low virulence and artificially attenuated cultures.

Experiments were carried out on white mice weighing 16-18 grams. The French preparation of cortisone acetate of the Roussel firm was used. Cortisone was administered to mice intramuscularly four hours prior to the test. At the end of this period the animals were injected subcutaneously with a suspension of a 48-hour culture of the tularemic or plague bacillus, or a diurnal (18-24 hours) culture of other causative agents in various doses, depending on the virulence of the strain.

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- 1) The work was carried out under the direction of N. G. Olsuf'yev and reported on 9 April 1960 at the Scientific Conference of the Central Antiplague Station.

A total of 20 strains of various bacterial species were studied.

The data obtained indicated that the mice were highly sensitive to recently isolated virulent strains of *Past. pestis*, and that cortisone had no substantial effect on the results.

An appreciable increase of sensitivity under the effect of cortisone was observed upon the testing of the plague vaccine strain EV. Upon testing of a fully virulent strain of *Past. tularensis* (No 9), no increase of sensitivity was noted in the animals following administration of cortisone.

Four pseudotuberculous strains were tested: two virulent ones (freshly isolated from wild rodents), one with reduced virulence, and one - an avirulent strain. In all of them, an increased sensitivity was noted under the effect of cortisone.

The authors also tested three *Listeria* strains, two *Past. multocida* strains, two *erysipelotrix* strains, and two *salmonella* strains. The results of tests with these causative agents were identical with those obtained with the pseudotuberculosis microorganism. In all cases, an increased sensitivity of mice to infection was noted following administration of cortisone.

Thus, cortisone considerably increased the sensitivity of mice to infections, whereas non-treated animals usually manifested only a moderate or low sensitivity. The enhanced sensitivity was expressed not only in the reduction of the minimal absolutely lethal dose (D_{01m}) but also in shortening of the period of seeding of organs and tissues with appropriate causative agents, which thus facilitated and accelerated the staging of a laboratory diagnosis.

This preparation can be employed in a number of cases as a bio-test, since it sharply increases the sensitivity of white mice to vaccine strains, including those of plague and tularemia microorganisms. As regards fully virulent cultures of plague and tularemia, to which white mice manifest high sensitivity even without cortisone, the administration of this preparation by the authors caused no increase in mortality (tularemia) or modified it but slightly (plague).

Rodents (*Rhombomys opimus*, *Mus musculus*, and in a few instances *Citellus pygmaeus*), were investigated under natural nidi conditions during an outbreak of plague epizooty among them, for the purpose of verification of the expediency of using cortisone-treated white mice for biological tests. The results of these tests showed no particular advantages of the laboratory diagnosis of plague in wild rodents in using cortisone-treated white mice for biological tests.

Conclusions

1. Under experimental conditions cortisone considerably increased the sensitivity of white mice to pseudotuberculosis, listerellosis, pasteurellosis, erysipeloid, and salmonellosis; therefore, cortisone-treated white mice can be employed as a very sensitive biotest in carrying out corresponding analyses.

2. Cortisone markedly increased the sensitivity of white mice to the vaccine culture of plague and tularemia microorganisms and thus, can be utilized in individual experimental investigations.

3. In experiments with highly virulent tularemia and plague cultures, to which white mice are highly sensitive even without cortisone, no further increase has been observed in the sensitivity of animals to infection (tularemia), or their sensitivity increased only slightly (plague).

4. Upon examination of wild rodents in the natural plague foci, no advantages could be elicited from the use of cortisone-treated white mice for biological tests.

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